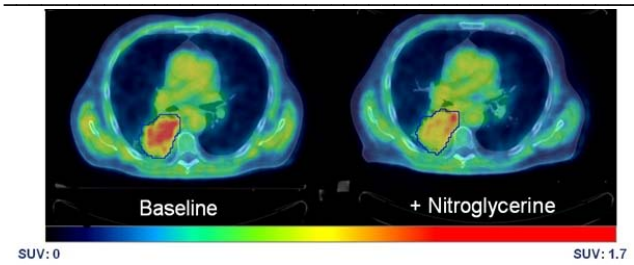

Figure 1: Example of HX-4 uptake in lung tumour before and after nitroglycerin.

Conclusion: Nitroglycerin causes a significant decrease in the hypoxic fraction and hypoxic volume of a majority of hypoxic non-small cell lung cancer tumours and metastatic lymph nodes. This promising result encourages further investigation of nitroglycerin as a sensitizing agent in a selected population. CT-based perfusion studies and lab experiments (data not shown) suggest this effect is mediated by an inhibition of mitochondrial respiration rather than a vascular effect.

OC-0130

Biomarker-based hypoxia-adapted radiochemotherapy: preclinical study in HPV+/- H&N cancer xenografts

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Purpose or Objective: Previous *in vivo* experiments demonstrated that hypoxia and perfusion determined during fractionated RT are associated with local tumour control (LC) in human head and neck squamous cell carcinoma (hHNSCC). In a randomized clinical trial Nimorazole improved LC and survival of patients with HNSCC treated with RT. Biomarker studies using tumour material from this trial indicate that hypoxic tumours predominantly benefit from nimorazole, supporting a predictive value for hypoxia assessment. However, this has not been prospectively evaluated for radiochemotherapy (RCTx) which represents the current standard of care in locally advanced head and neck cancer. The hypothesis of the ongoing study is that the microenvironmental parameters are also predictive for response to hypoxic cell sensitizing with nimorazole in combination with RCTx.

Material and Methods: We studied 8 different human HPV-negative and -positive HNSCC in a nude mice xenograft model. Irradiation was performed with 30 fractions (fx) in six weeks combined with weekly cisplatin (3 mg/kg i. p.). Nimorazole (0.3 mg/g i. p.) was applied before each irradiation and was started with the first fx or after 10 fx. Effect of nimorazole was quantified as LC 120/180 days after irradiation. For histological evaluation tumours were excised unirradiated or after 10 fx with and without nimorazole. Using quantitative image analysis, microenvironmental parameter such pimonidazole hypoxic volume (pHV), relative vascular area (RVA) and perfused fraction of vessels (PF) were determined.

Results: The data of the cell lines show pronounced heterogeneity in the effect of nimorazole on local tumour control after fractionated radiochemotherapy. Nimorazole significantly improved local tumour control in four of the eight tumours. In the two responder models FaDu and SAS, nimorazole was equally effective when given from start of radiochemotherapy or after 10 fx. The treatment with both, RCTx and the application of nimorazole and cisplatin were well tolerated by the animals. Furthermore, pHV was significantly reduced after 10 fx RCT with and without nimorazole in all four responder models in contrast to the non-responders.

Conclusion: Apparently, the decrease of pHV after the first fractions of RCTx has potential as a predictive biomarker for LC for combination of RCTx with nimorazole and should therefore be further evaluated in experimental FMISO analysis and also in clinical trials using hypoxic cell radiosensitisation during RCTx.

OC-0131

miR-875-5p enhances radiation response of prostate cancer cells via EGFR suppression

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Purpose or Objective: There is increasing interest in defining a functional association between miRNAs, endogenous small non-coding RNA molecules that negatively regulate gene expression, and tumor radiation response, with the aim of rationally designing miRNA-based strategies to increase patient radiosensitivity. In this study, we investigated for the first time the ability of *miR-875-5p,* a miRNA the role of which in human cancer has not been so far investigated, to enhance the radiation response of prostate cancer (PCa) cells.

Material and Methods: The search for *miR-875-5p* targets relevant to radiation response was carried out by prediction algorithms and confirmed by the luciferase assay. *miR-875-5p* reconstitution by miRNA mimics in PCa cell lines (DU145 and PC-3) was used to elucidate its biological role. Radiation response in miRNA-reconstituted and control cells was assessed by clonogenic assay, immunofluorescence-based detection of nuclear γ-H2AX foci and single-cell electrophoresis comet assay.

Results: EGFR was predicted by 6 different algorithms and confirmed by luciferase assay as a direct target of *miR-875-5p.* Given the role of EGFR in determining tumor cell resistance to ionizing radiation by promoting epithelial-to-mesenchymal transition (EMT) and enhancing DNA-dependent protein kinase activity and DNA damage repair, we assessed whether *miR-875-5p* reconstitution in PCa cells was able to counteract EGFR-mediated radio-resistance. Indeed, miRNA ectopic expression significantly increased the sensitivity of both DU145 and PC-3 cell lines to radiation, as indicated by the reduced clonogenic cell survival. Consistently, the kinetics of accumulation and repair of γ-H2AX nuclear foci showed that the resolution of foci was significantly slower in *miR-875-5p* reconstituted cells compared to control cells. In addition, when a more direct assessment of radiation-induced DNA damage and repair at the single cell level was performed by the comet assay, DNA comet tail moments were found to be significantly extended in *miR-875-5p* reconstituted cells compared to control cells, confirming the ability of the miRNA to impair DNA repair processes. Ectopic expression of *miR-875-5p* in PCa cells was also able to counteract EMT as indicated by changes in cell morphology, marked cytoskeleton architecture rearrangements, reduced migration ability and increased mRNA and protein levels of E-cadherin and ß-catenin, the two most important molecular players in the EMT process.

Conclusion: Overall, results from this study support the clinical interest in developing a novel therapeutic approach for PCa based on *miR-875-5p* reconstitution to increase response to radiotherapy.

OC-0132

FoxO proteins and non-functional p53 determine stemness and radiosensitivity of GBM-stem cells

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Purpose or Objective: Dual inhibitors of PI3K and mTOR do not efficiently radiosensitize glioblastoma multiforme stem cells (GBM-SCs). However, p53-proficient GBM-SCs are more